Histological and biomechanical study of impacted cancellous allografts with cement in the femur - a canine model -

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Introduction

Impaction allografting of bone has been used successfully as a technique to reconstitute bone loss in the femur in revision total hip arthroplasty (THA) since the pioneering work of Exeter, England (5). The procedure involves progressive compaction of morselized cancellous bone chips into the femoral canal. The prosthesis is then cemented in place, creating a three-layer composit of implant, cement and graft. Recently successful long-term results of this procedure have been reported (7)(21). However, the short-term results have varied considerably. Subsidence of the femoral stem occurs in 0-86% of the patients (11)(14), and the failure rates in terms of mechanical loosening vary from 0-4% after 5 years (11)(25), to 10% already after 1 year (4) and 19% after 5 years (28).

Despite the widespread clinical use of impaction bone allografts, little is known about the physiological events that place in the graft bed.

Nelissen et al. obtained biopsy specimens from the proximal part of the femur at the time of removal of trochanteric wires from four patients eleven to twenty-seven months after revision hip arthroplasty with cement and impaction grafting. They reported three ill defined zones were identified histologically: an inner zone consisting of bone cement, fibrous tissue, and partially necrotic trabeculae with evidence of bone-remodeling; a middle zone consisting of viable trabeculae bone and formation of so-called neocortex; and an outer zone consisting of viable cortex (16).

Linder studied 6 whole postmortem femurs and 8 femoral biopsy histologically, 3 months to 8 years after cancellous impaction grafting with a cemented stem. He reported that one patient showed complete bony restitution and the others still had varying amounts remaining graft in the neo-medullary cavity, even after 8 years (12).

Ullmark et al. reported the histological findings of 31 tissue samples from 21 cases in 19 different patients taken 1 to 48 months after revision arthroplasty and impaction grafting in the hip. One month after surgery, a fibrous stroma and some newly formed woven bone were found in the graft bed. After 4 months, many of the dead trabeculae in the graft bed had layers of living bone and osteoid in all samples. In the proximal end of the femur examined after 48 months, a significant proportion of the graft bone remained dead, whereas in the rest of the femur, the bone healing was complete (26).

These clinical studies show a slow in- and on-growth of new bone onto the surfaces of the allograft, with a very slow rate of graft resorption. Soft tissue ingrowth seems to precede bone formation. There have been no reports on consecutive and quantitative histological evaluation of the allograft bone resorption process.

We therefore performed a quantitative and consecutive histological evaluation using bone morphometry during the early postoperative period in a canine model of impacted cancellous femoral allografting. In addition, we performed load tests at 8 weeks after surgery.

Materials and Methods

Materials

The prosthesis had a collarless polished titanium alloy tapered stem with a head diameter of 15 mm. Twelve Beagle dogs (50 weeks old) weighing about 10 kg were used. Cancellous bone grafts were obtained aseptically from the femoral condyles of other beagles. The grafts were frozen at -80 ° C until implantation, and were allowed to thaw at room temperature before use. The storage time was 3 months. Each bone chip had a diameter of about 3 mm.

Operative procedure

The femoral head was dislocated using the postero-lateral approach under the general anesthesia. After the femoral head had been resected, the cancellous bone in the femoral marrow was completely excised and the femoral cortex was reamed without causing any fractures. The cancellous allograft material (about 1.5 g/cm^3 of bone defect) was then impacted in the medullary cavity using a tamper that was 1 mm larger than the stem of the prosthesis, and the stem was cemented in place (Fig. 1). The cement used was Zimmer regular PMMA cement (Zimmer Inc., Warsaw, IN, USA). The cement was injected in the neo-medullary canal from the femoral neck using a syringe and the prosthesis was inserted. The position of the prosthesis was mostly neutral in the coronal plane and 20 degrees anteverted in axial plane. A hemipelvic plaster cast was applied for 2 weeks postoperatively.

There were no surgical or postoperative complications such as infections or dislocations.

Histological evaluation

Calcein (200 mg) was injected twice intramuscularly in 12 animals (four animals each at 7, 8 weeks, 15,16 weeks, and 49,50 weeks postoperatively) with a six-day washout period between the two injections. The femora were removed after the animals had been sacrified by an overdose of pentobarbiturate at 8 weeks, 16 weeks, and one year postoperatively. After a prosthesis was removed, undecalcified sections were prepared with Villaneuva staining at a proximal site (at the lesser trochanteric level) and a distal site (1 cm proximal from the tip of the prosthetic stem) for histological evaluation of the bone grafts (Fig. 2-a,b). Image analysis software (Osteoplan, Carl Zeiss, NY, USA) was used to determine the mineral apposition rate and the bone formation rate in the proximal and distal sites of the intramedullary graft. ***The mineral apposition rate and the bone formation rate were measured at twenty sites of one section, and the mean value was calculated (18,19).***

Load test and computation of strain

We used 8 animals (impacted cancellous allograft with cement group; n=4, only cemented group; n=4) at 8 weeks after surgery for strain evaluation. In these animals, radiographs of each hip joint were taken immediately following surgery and at 8 weeks after surgery. These radiographs were used for observation only. The femurs for mechanical evaluation were freshly harvested and stored at -80 ° C until testing.

Rosette strain gauges (Kyowa KFG-2-120-D-17-11 L3M, Kyowa Dengyo, Tokyo, Japan) (Fig. 3) were used. The three gauges were mounted so that in counterclockwise direction they were gauge (a), gauge (b) and gauge (c), with gauge (b) always parallel to the long axis direction of the femur.

After thawing, the femurs were resected just above the condyles. The femoral axis was placed at an angle of 15 degrees to vertical in the coronal plane, and the load was introduced vertically onto the head. It was then placed vertically in the sagittal plane and fixed with rapid drying cement. We prepared a device for pressing the femoral head, which was made of an iron cup 15 mm in diameter. Strain gauges were attached to the femoral surface with cyanoacrylate (Allon alpha, Toa Gosei, Tokyo, Japan) in 8 locations to determine the strain on the proximal and distal parts of the stem (Fig. 4). Load was applied to the head by advancing the crosshead of an axial load cell (Shimadzu Autograph AGS-1000-A, Shimadzu, Tokyo, Japan) at rate of 0.3 mm/s until 30 kgf was reached (Fig. 5).

Strain values εa, εb, and εc obtained by rosette strain gauges (a), (b) and (c), respectively, were substituted in the formula and the maximum and minimum strain values were obtained as follows:

$$\varepsilon 1 = 1/2 \{ \varepsilon a + \varepsilon c + \sqrt{2} [(\varepsilon a - \varepsilon b)2 + (\varepsilon b - \varepsilon c)2]\}$$

$$\varepsilon 2 = 1/2 \{ \varepsilon a + \varepsilon c - \sqrt{2} [(\varepsilon a - \varepsilon b)2 + (\varepsilon b - \varepsilon c)2]\}$$

where $\varepsilon 1$ is a maximum principal strain, $\varepsilon 2$ is a minimum principal strain.

Loading tests were conducted one time. Statistical analysis was done using only the highest principal strain ***(6).***

The protocol of this experimental study was approved by the Ethics Committee of Hiroshima University, and the principles of laboratory animal care (3) were observed.

Statistical analysis

Statistical analysis was performed using the Mann-Whitney U test and significant differences were noted at $p < 0.05$.

Results

1) Microscopic findings of undecalcified specimens

At 8 weeks postoperatively, new bone had formed in the proximal site, especially adjacent to the host cortical bone. In this area, creeping substitution consistent with gradual graft incorporation was found.

Vascularized fibrous tissue was present around some of the incorporating bone graft fragments. At the distal site creeping substitution was scanty (Fig. 6-a, b). At 16 weeks after surgery, active osteogenesis was also observed at the distal site and new bone trabeculae were found. At one year postoperatively, new bone trabeculae had formed extensively in both the proximal and distal sites (Fig. 7-a,b). However, small amounts of allograft in the vascularized fibrous tissue remained in both the proximal and distal sites at sites in contact with the cement (Fig. 8).

2) Bone morphometry

The mineral apposition rate and the bone formation rate were both significantly higher in the proximal site than in the distal site at 8 weeks postoperatively, but no significant difference was seen between these rates the proximal and distal sites after 16 weeks or one year (Tables 1 and 2). In the proximal site, the mineral apposition rate showed a significant decrease at 16 weeks when compared with that at 8 weeks postoperatively, but no significant differences were observed at other times (Table 1). No significant difference was found between in the bone formation rates in the proximal site at 8 weeks and 16 weeks after surgery, though there was a significant decrease in the bone formation rate at one year compared with that at 16 weeks postoperatively. The bone formation rates in distal site significantly increased at 16 weeks than at 8 weeks and decreased at 1 year than at

16 weeks after surgery (Table 2).

3) Load test

Radiographically, all implants appeared to be well fixed, with no obvious signs of subsidence before the load test (Fig. 9-a,b).

On the proximal side of the femur, there was no significant difference of strain value between the impaction grafted group and only cemented group (Table 3). Compressive strain was observed on the medial side (gauge ①) and anterior side (gauge ③) and tensile strain was observed on the lateral side (gauge ②) and posterior side (gauge ④)

Also on the distal side of the femur, No significant difference of strain value was observed between both groups (Table 4). Compressive strain was observed on the medial side (gauge ⑤) and anterior side (gauge ⑦) and tensile strain was observed on the lateral side (gauge ⑥) and posterior side (gauge ⑧).

Discussion

The short-term success of revision arthroplasty with impaction grafting is related to the initial stability of the construct (1). In the present study, there was no significant difference of strain value between the impaction grafted group and only cemented group at eight sites of the femur. Our experiment showed compressive strain at medial sites and tensile strain at lateral sites in both groups. These results are consistent with other biomechanical analysis using primary THA models (10,17), thus the initial stability of the construct was accomplished in our experimental model.

Histological evaluation of the process of replacement of bone graft by new bone in a small number of clinical cases (12)(13)(16)(24)(26) or in animal studies (20)(23) has shown that the process can be summarized as follows: <1> the regenerated cortical bone layer comes into contact with the host cortical bone, <2> a boundary layer forms at the site of contact between the osteoid and cement mixed with fibrous tissue, and <3> a deep layer of necrotic grafted trabeculae is incorporated in the cement and soft tissue or osteoid connected to the surrounding bone. However, these previous studies cannot really be considered as consecutive and quantitative evaluations. The present study showed the progression of bone replacement from the proximal site to the distal site of the femur in the longitudinal direction, and from the host cortical bone to the cement in the transverse direction.

The limitation of this study is that the bone morphometry of contralateral femur was not evaluated. Monier-Faugere et al. reported that the mineral apposition rate and the bone formation rate of Beagle dogs' iliac bone were 0.48 mm/yr and 6.3 mm^3/cm^2/yr, respectively (15). In this study, the mineral apposition rate of the distal site at eight weeks after surgery only revealed a lower value and the other rates revealed higher values than those of Monier-Faugere et al. On the other hand, the bone formation rate of the proximal site at eight weeks after surgery only revealed a higher value and the other rates revealed lower values than those of them.

Considering microscopic findings and bone morphometry, we concluded that biological replacement of the grafted bone by new bone settled at one year after surgery in this experimental model. However, the process was not completed.

Studies using positron emission tomography have shown that the initial increase in blood flow and bone remodeling following surgery reduces to baseline levels by one year, implying that incorporation is complete by this stage (22)(27). We have previously evaluated the change in appearance of impacted cancellous allograft in the femur over time on scintigraphic scans and reported that remodelling of the grafted bone of the femur is still incomplete, particularly at the greater trochanter, lesser trochanter, and stem tip even after 4 years postoperatively (8). The reduction of vascularization and bone formation with the positron emission tomography technique does not necessarily mean complete bone replacement of bone allograft in a clinical case, but may mean the frustration of bone replacement.

In the clinical cases, it is speculated that the new bone formation proceed from the proximal part to the distal site of the femur in the long axis as well as from the host cortical bone to the cement layer. However, since the host cortical bone in patients is not as healthy as that in Beagle models and since the bone defects in patients are larger, it is assumed that replacement by new bone in patients will take longer. Especially in a case with large bone loss, it is necessary to take account of the operative indication for this technique.

Detailed studies on the immunological response to cryopreserved bone (9), sizes of grafted bone fragments, thicknesses of bone grafts, using bone-graft substitutes such as hydroxyapatite (2) technique of impaction, and differences in the replacement process depending on the timing of weight bearing time (1) are required in the future to establish this method as a safe surgical procedure.

Acknowledgement

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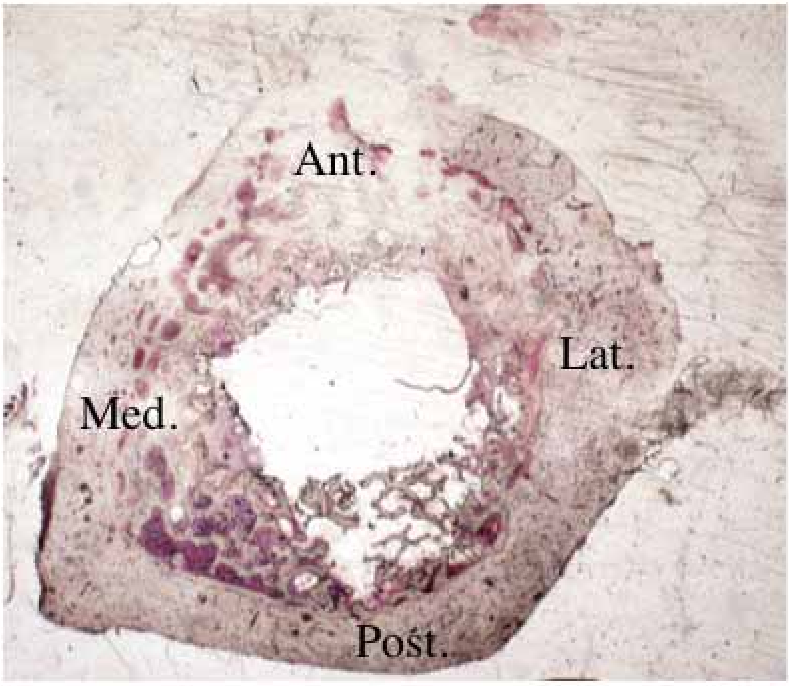
Figure legends

Fig. 1. Longitudinal cross-sectioned view after surgery.

Stem: Collarless polished titanium alloy tapered stem with a head diameter of 15 mm.



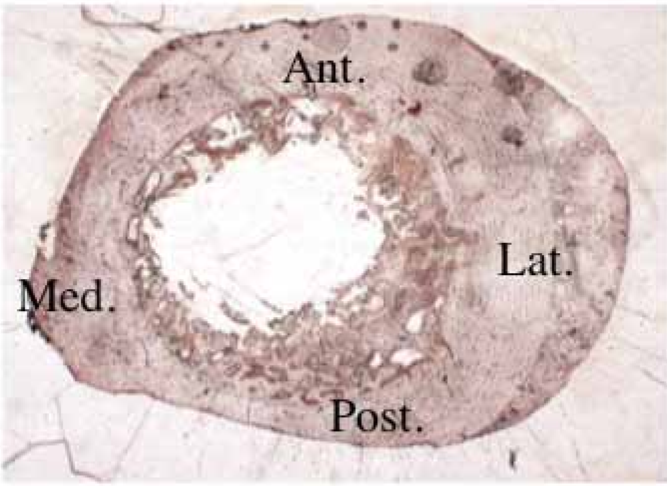


Fig. 2. Undecalcified cross-section for histological evaluation.

The thickness of section is approximately 20 μm.

a: Proximal site, b: Distal site

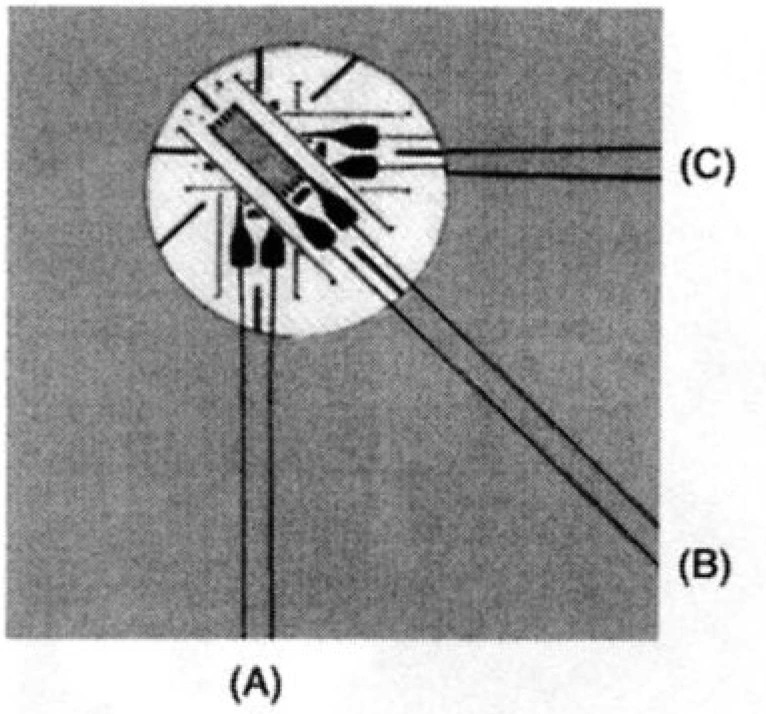


Fig. 3. Strain gauge; gauge element a (A), gauge element b (B), gauge element c (C)

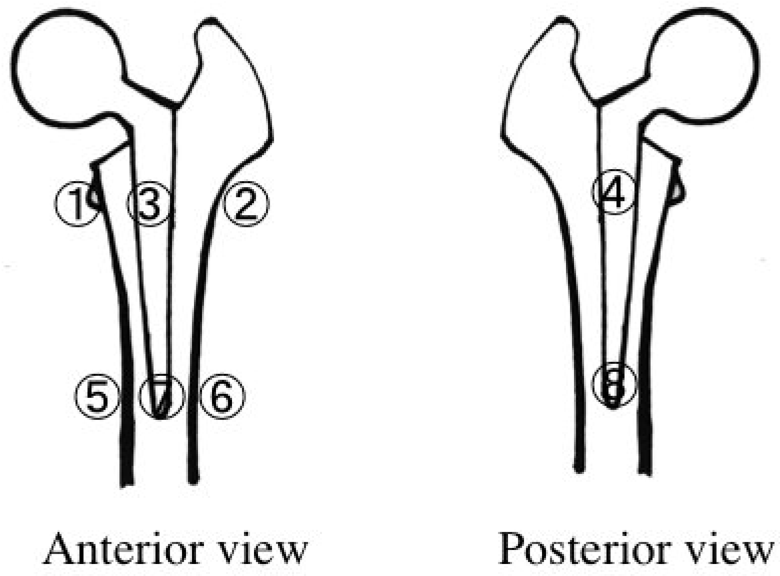


Fig. 4. Sites of attachment of strain gauges.

①; Medial site of proximal femur at the lesser trochanter level

②; Lateral site of proximal femur at the lesser trochanter level

③; Anterior site of proximal femur at the lesser trochanter level

④; Posterior site of proximal femur at the lesser trochanter level

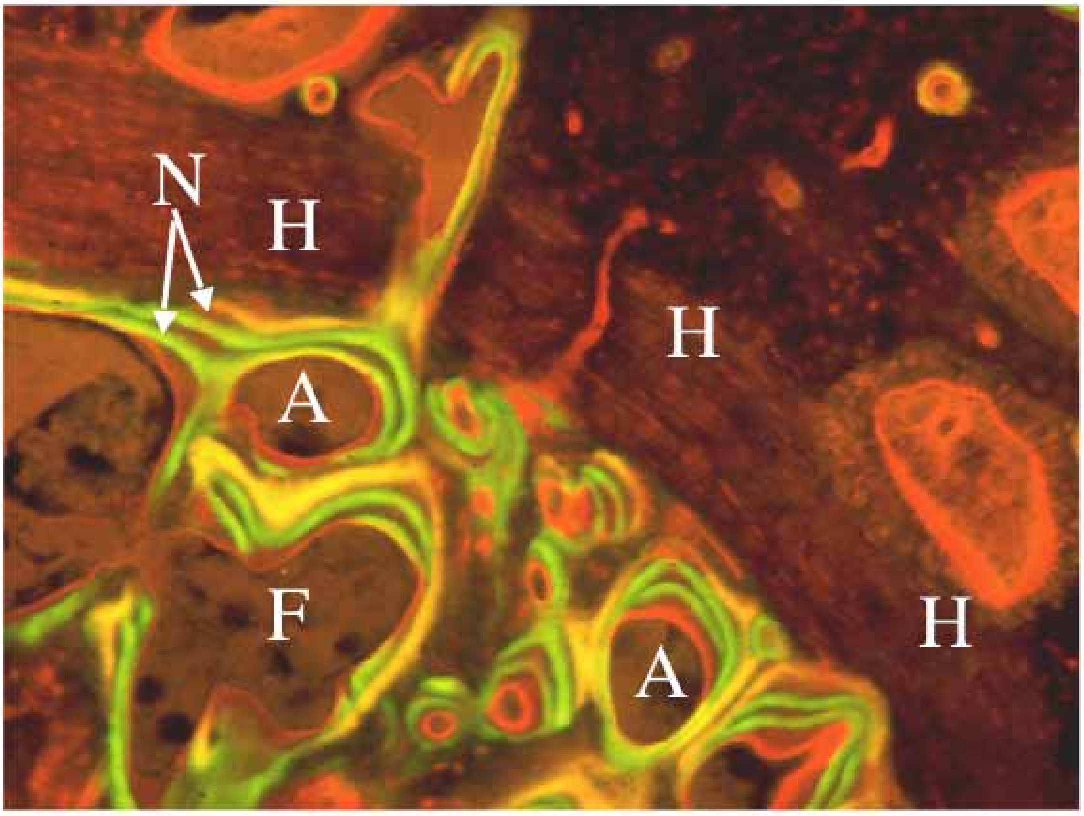
⑤; Medial site of distal femur at the stem tip level

⑥; Lateral site of distal femur at the stem tip level

⑦; Anterior site of distal femur at the stem tip level

⑧; Posterior site of distal femur at the stem tip level

Fig. 5. Mechanical testing system.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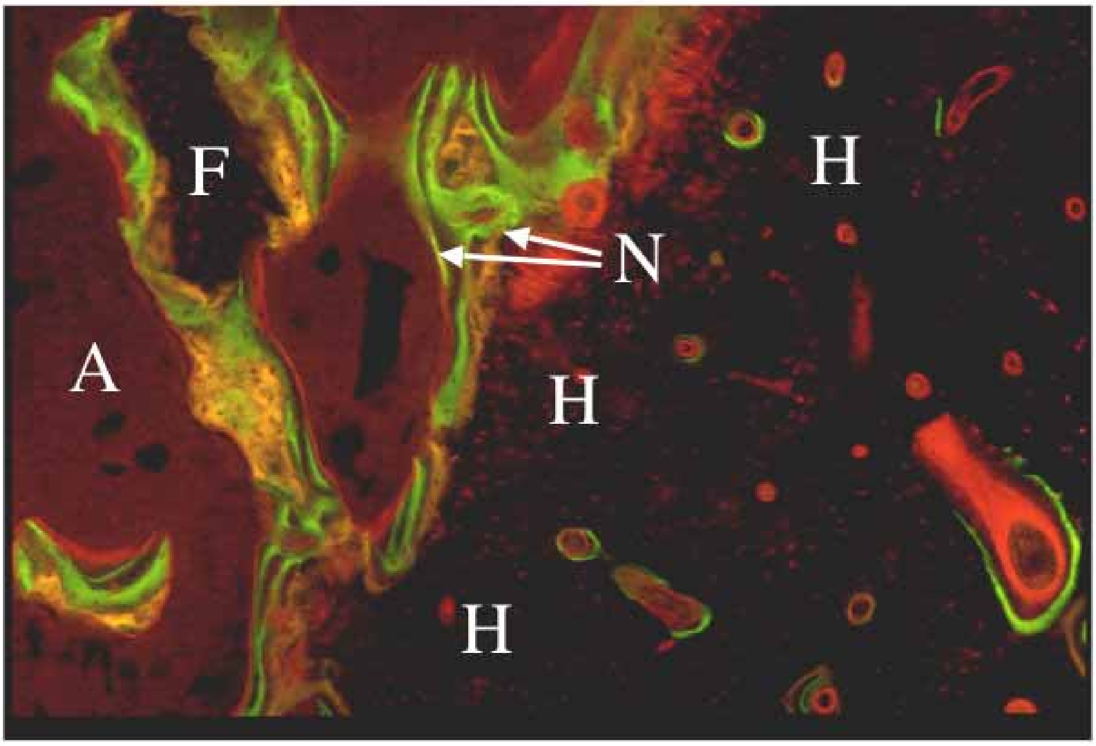
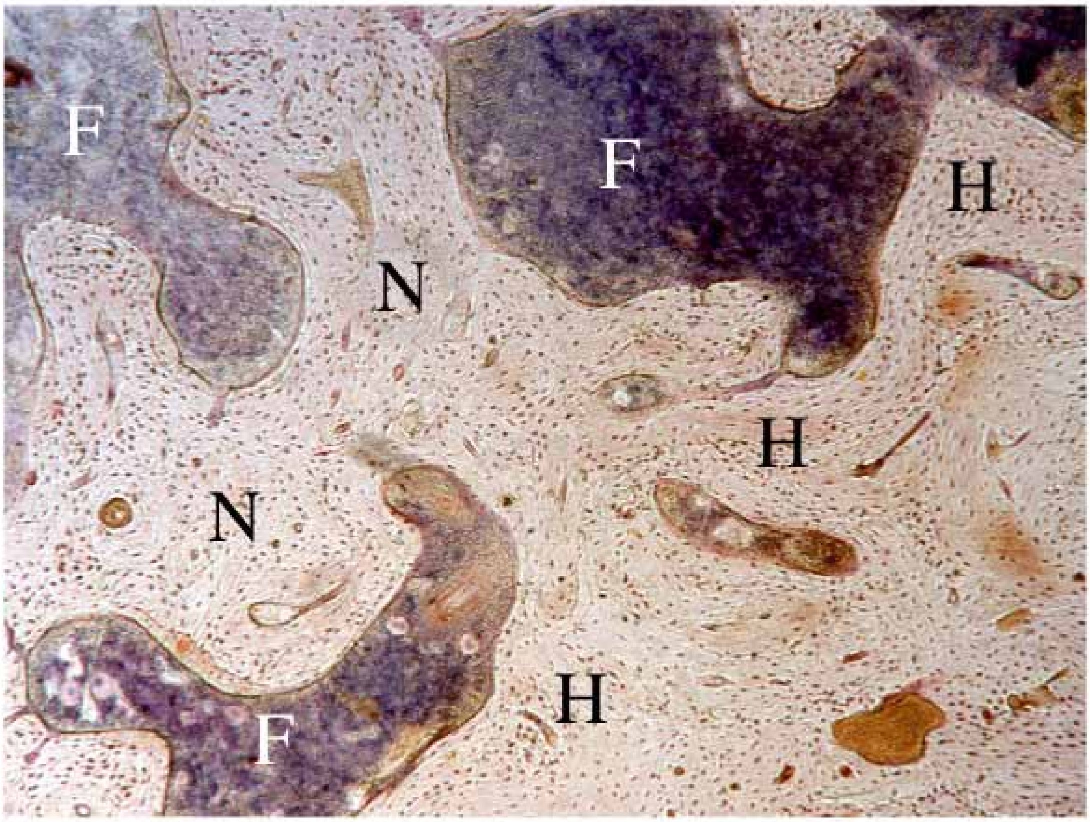


Fig. 6. Eight weeks after surgery. (Villanueva stain, x100, under fluorescent light)

a : In the proximal site, calcein green fluorescence is clearly seen and new bone are clearly formed .

b : In the distal site, new bone are only partly formed.

H: Host cortical bone, N: New bone, F: Vascularized fibrous tissue, A: Allograft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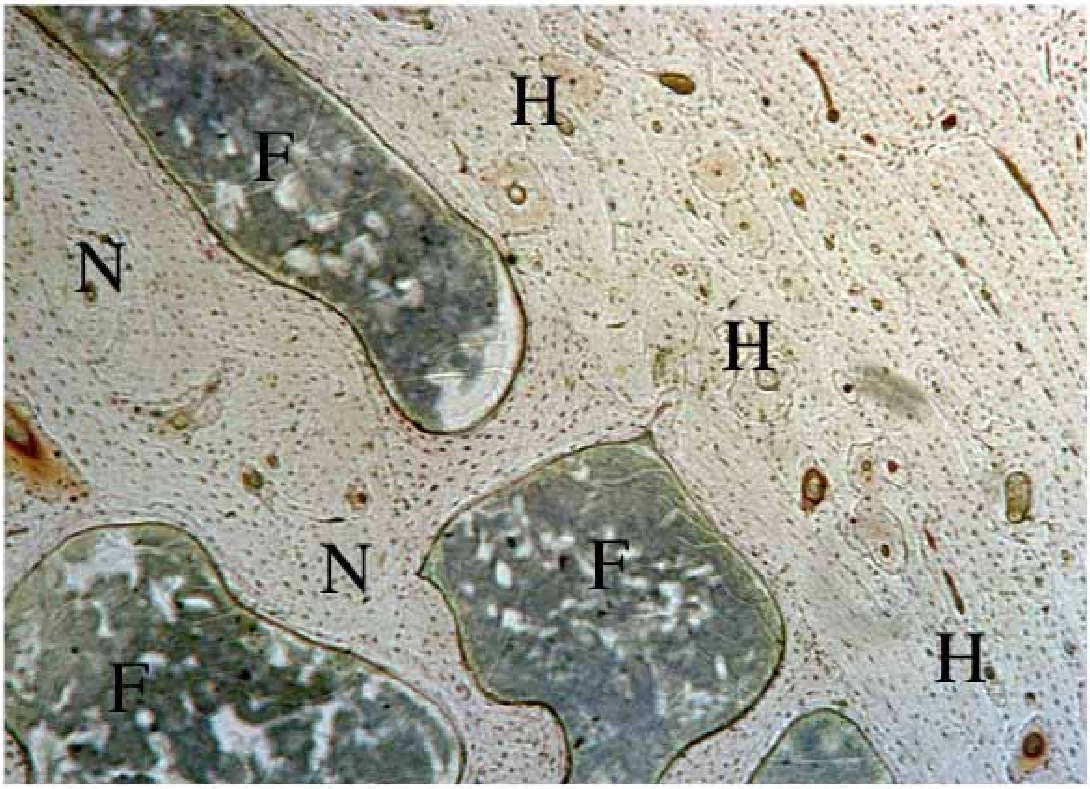


Fig. 7. One year after surgery. (Villanueva stain, x50, under incandescent light)

a : Proximal site.   b : Distal site.

Nearly normal trabeculae are formed at one year after surgery.

H: Host cortical bone, N: New bone

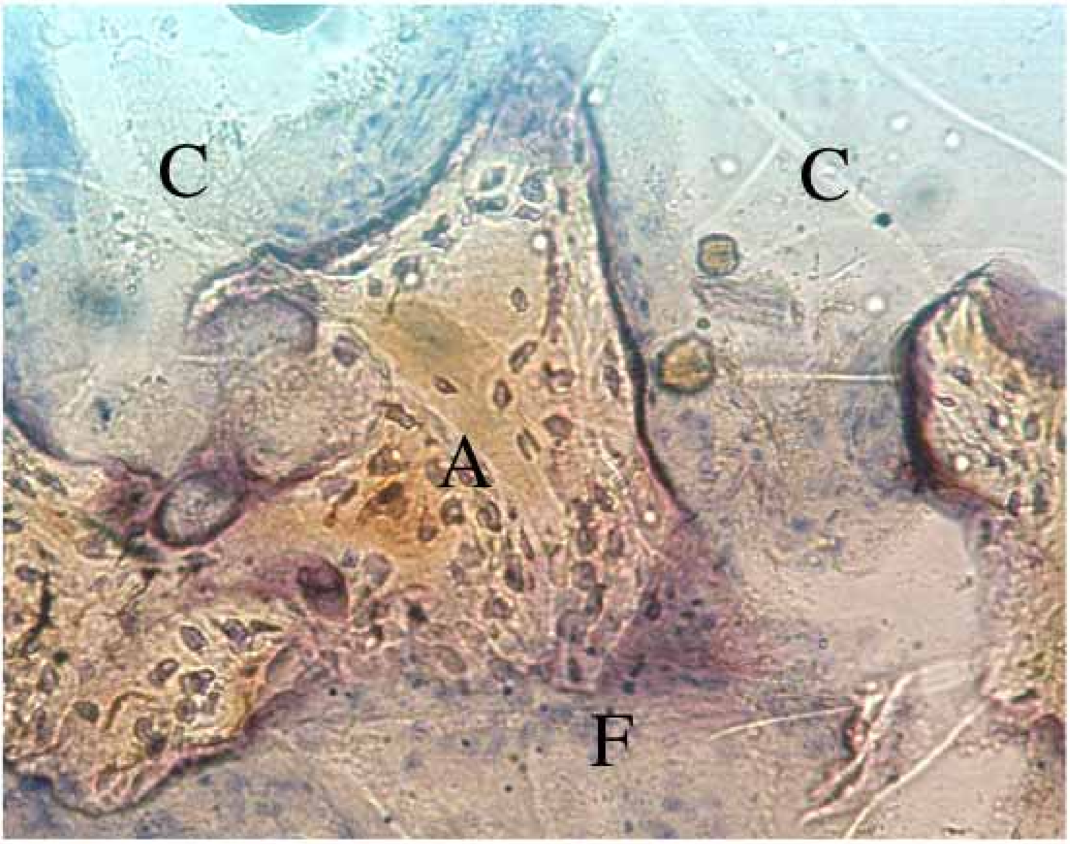
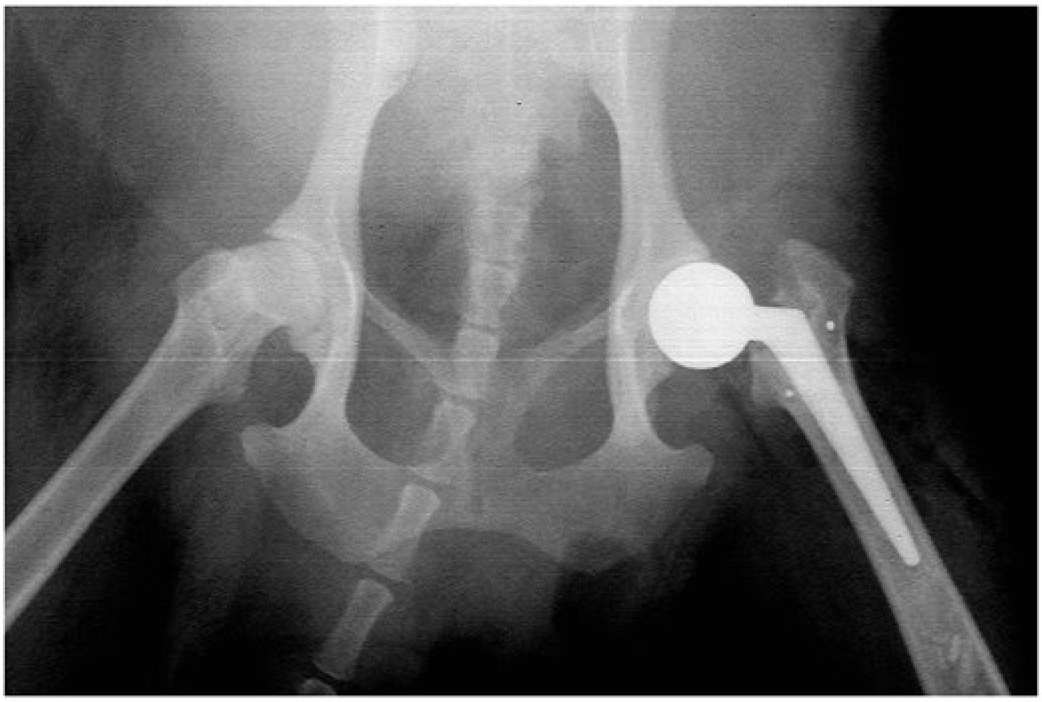


Fig. 8. Distal site at one year after surgery. (Villanueva stain, x50, under incandescent light light)

C: Cement, F: Vascularized fibrous tissue, A: Allograft



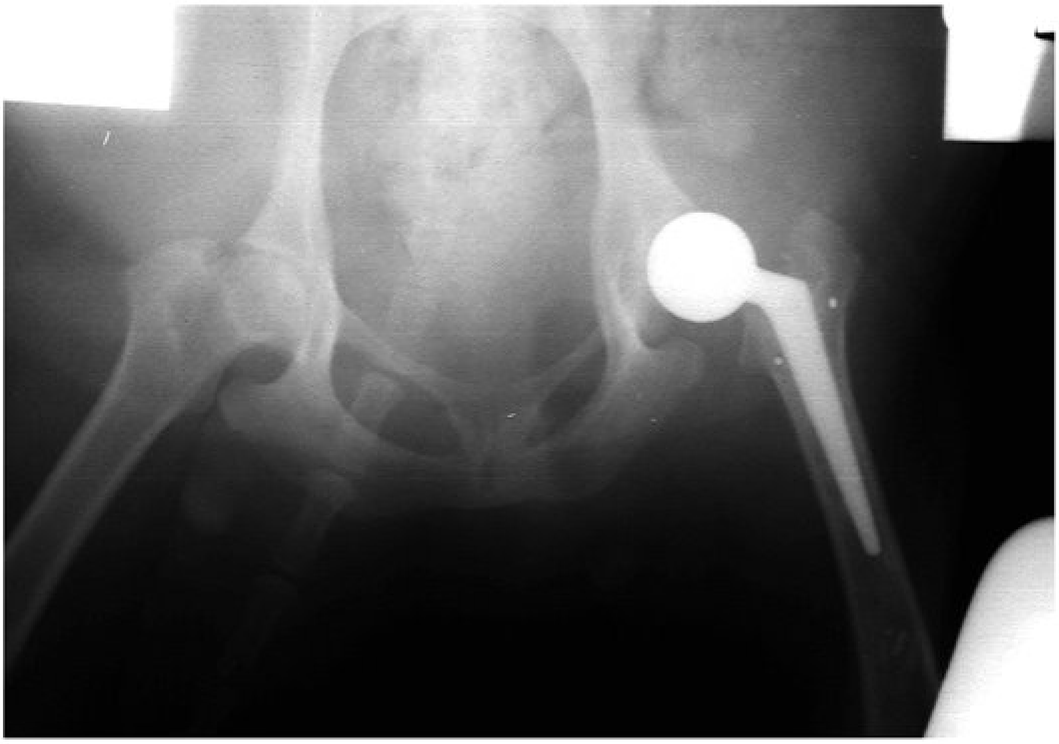


Fig. 9. Radiographs

a: After surgery. b: 8 weeks after surgery.

Table 1. Mineral apposition rate (mm/year)

Canine No.	Proximal site		Distal site
8 weeks after surgery			
No.1	1.0		0.73
2	0.85	*	0.26
3	0.98		0.35
4	1.1		0.52
	Mean 0.98 S.D. 0.10		Mean 0.47 S.D. 0.21
	*		N.S.
16 weeks after surgery			
5	0.64		0.63
6	0.53	N.S.	0.64
7	0.63		0.63
8	0.57		0.67
	Mean 0.59 S.D. 0.05		Mean 0.64 S.D. 0.02
	N.S.		N.S.
One year after surgery			
9	0.54		0.63
10	0.69	N.S.	0.55
11	0.61		0.62
12	0.61		0.59
	Mean 0.61 S.D. 0.06		Mean 0.60 S.D. 0.04

* : $p < 0.05$ Mann-Whitney U test

Table 2. Bone formation rate ($mm^3/cm^2/year$)

Canine No.	Proximal site		Distal site
8 weeks after surgery			
No.1	8.9		3.2
2	6.7		0.52
3	7.6	*	1.6
4	7.2		0.67
	Mean 7.6 S.D. 0.94		Mean 1.5 S.D. 1.2
	N.S.		*
16 weeks after surgery			
5	4.8		3.0
6	7.6	N.S.	8.1
7	5.9		4.4
8	6.2		5.3
	Mean 6.1 S.D. 1.2		Mean 5.2 S.D. 2.2
	*		*
One year after surgery			
9	1.2		3.1
10	2.8	N.S.	2.3
11	2.0		1.8
12	1.8		2.5
	Mean 2.0 S.D. 0.66		Mean 2.4 S.D. 0.54

* : $p < 0.05$ Mann-Whitney U test

Table 3. Strain value (με) at the proximal part

Grafted group	Canine No.			Control group	Canine No.	
① Medial	No. 15	-809	NS	① Medial	No. 19	-963
	16	-1007			20	-794
	17	-674			21	-598
	18	-751			22	-649
	Mean -810 S.D. 142				Mean -751 S.D. 164	
② Lateral	No. 15	365	NS	② Lateral	No. 19	197
	16	408			20	110
	17	537			21	439
	18	310			22	346
	Mean 405 S.D. 96.7				Mean 273 S.D. 147	
③ Anterior	No. 15	-353	NS	③ Anterior	No. 19	-296
	16	-409			20	-406
	17	-589			21	-618
	18	-553			22	-349
	Mean -476 S.D. 113				Mean -417 S.D. 141	
④ Posterior	No. 15	209	NS	④ Posterior	No. 19	137
	16	128			20	229
	17	269			21	198
	18	306			22	98
	Mean 228 S.D. 77.7				Mean 166 S.D. 59.0	

Mann-Whitney U test

Table 4. Strain value (με) at the distal part

Grafted group	Canine No.			Control group	Canine No.	
⑤ Medial	No. 15	-1109		⑤ Medial	No. 19	-978
	16	-1207			20	-825
	17	-774	NS		21	-690
	18	-751			22	-732
	Mean -960 S.D. 232				Mean -806 S.D. 128	
⑥ Lateral	No. 15	427		⑥ Lateral	No. 19	334
	16	540			20	298
	17	628	NS		21	523
	18	242			22	417
	Mean 459 S.D. 167				Mean 393 S.D. 100	
⑦ Anterior	No. 15	-252		⑦ Anterior	No. 19	-198
	16	-139			20	-376
	17	-248	NS		21	-532
	18	-353			22	-328
	Mean -248 S.D. 87.4				Mean -359 S.D. 138	
⑧ Posterior	No. 15	164		⑧ Posterior	No. 19	156
	16	97			20	353
	17	291	NS		21	294
	18	309			22	76
	Mean 215 S.D. 102				Mean 220 S.D. 126	

Mann-Whitney U test